09/245,749

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Search Results - Record(s) 1 through 10 of 13 returned.

☐ 1. Document ID: US 6281000 B1

L9: Entry 1 of 13 File: USPT Aug 28, 2001

US-PAT-NO: 6281000
DOCUMENT-IDENTIFIER: US 6281000 B1

TITLE: Method for the preparation of a viral vector by intermolecular homologous recombination

DATE-ISSUED: August 28, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Chartier; Cecile	Strasbourg	N/A	N/A	FRX
Degryse; Eric	Strasbourg	N/A	N/A	FRX

US-CL-CURRENT: 435/252.33; 424/184.1, 424/199.1, 424/200.1, 435/252.3, 435/320.1, 435/69.1, 435/69.3, 435/7.2, 514/44, 530/300, 530/350, 536/23.1, 536/23.7, 536/23.72

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw. Desc | Image

☐ 2. Document ID: US 6110735 A

L9: Entry 2 of 13 File: USPT Aug 29, 2000

US-PAT-NO: 6110735
DOCUMENT-IDENTIFIER: US 6110735 A

TITLE: Method for the preparation of a viral vector by intermolecular homologous recombination

DATE-ISSUED: August 29, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Chartier; Cecile	Strasbourg	N/A	N/A	FRX
Degryse; Eric	Strasbourg	N/A	N/A	FRX

US-CL-CURRENT: 435/320.1; 424/233.1, 435/235.1, 435/252.1, 435/252.3, 435/252.33, 435/252.8, 435/91.4, 435/91.41, 514/44 , 530/350

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw. Desc | Image

☐ 3. Document ID: US 6077697 A

L9: Entry 3 of 13 File: USPT Jun 20, 2000

US-PAT-NO: 6077697
DOCUMENT-IDENTIFIER: US 6077697 A

TITLE: Artificial chromosomes, uses thereof and methods for preparing artificial chromosomes

DATE-ISSUED: June 20, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Hadlaczky; Gyula	Szamos	N/A	N/A	HUX
Szalay; Aladar A.	Highland	CA	N/A	N/A

US-CL-CURRENT: 435/6; 435/320.1, 435/325, 435/455, 435/468, 435/69.1, 435/91.1, 536/22.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | KWIC | Draw Desc | Image

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☐ 4. Document ID: US 6025155 A

L9: Entry 4 of 13 File: USPT Feb 15, 2000

US-PAT-NO: 6025155
DOCUMENT-IDENTIFIER: US 6025155 A

TITLE: Artificial chromosomes, uses thereof and methods for preparing artificial chromosomes

DATE-ISSUED: February 15, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Hadlaczky; Gyula	Szamos	N/A	N/A	HUX
Szalay; Aladar A.	Highland	CA	N/A	N/A

US-CL-CURRENT: 435/69.1; 435/320.1, 435/325, 435/455, 435/6

Full | Title | Citation | Front | Review | Classification | Date | Reference | KWIC | Draw Desc | Image

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☐ 5. Document ID: US 6015687 A

L9: Entry 5 of 13 File: USPT Jan 18, 2000

US-PAT-NO: 6015687
DOCUMENT-IDENTIFIER: US 6015687 A

TITLE: Apoptosis-modulating proteins, DNA encoding the proteins and methods of use thereof

DATE-ISSUED: January 18, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kiefer; Michael C.	Clayton	CA	N/A	N/A
Barr; Philip J.	Berkeley	CA	N/A	N/A

US-CL-CURRENT: 435/69.1; 435/320.1, 435/325, 435/455, 435/91.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | KWIC | Draw. Desc | Image

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☐ 6. Document ID: US 5998131 A

L9: Entry 6 of 13 File: USPT Dec 7, 1999

US-PAT-NO: 5998131
DOCUMENT-IDENTIFIER: US 5998131 A

TITLE: Screening methods for the identification of compounds capable of abrogating BaK-BHRF-1 protein interactions

DATE-ISSUED: December 7, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Barr; Philip J.	Berkeley	CA	N/A	N/A
Kiefer; Michael C.	Clayton	CA	N/A	N/A

US-CL-CURRENT: 435/5; 424/230.1, 424/233.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | KWIC | Draw. Desc | Image

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☐ 7. Document ID: US 5972614 A

L9: Entry 7 of 13 File: USPT Oct 26, 1999

US-PAT-NO: 5972614
DOCUMENT-IDENTIFIER: US 5972614 A

TITLE: Genome anthologies for harvesting gene variants

DATE-ISSUED: October 26, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Ruano; Gualberto	New Haven	CT	N/A	N/A
Bentley; Kevin L.	Madison	CT	N/A	N/A
Ruddle; Frank H.	New Haven	CT	N/A	N/A

US-CL-CURRENT: 435/6; 435/254.11, 435/254.2, 435/255.1, 435/320.1, 435/440

Full | Title | Citation | Front | Review | Classification | Date | Reference KWIC | Draw Desc | Image

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☐ 8. Document ID: US 5770443 A

L9: Entry 8 of 13 File: USPT Jun 23, 1998

US-PAT-NO: 5770443
DOCUMENT-IDENTIFIER: US 5770443 A

TITLE: Apoptosis-modulating proteins, DNA encoding the proteins and methods of use thereof

DATE-ISSUED: June 23, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kiefer; Michael C.	Clayton	CA	N/A	N/A
Barr; Philip J.	Berkeley	CA	N/A	N/A

US-CL-CURRENT: 435/325; 435/320.1, 435/69.1, 435/91.2, 536/23.1, 536/23.5, 536/24.31

Full | Title | Citation | Front | Review | Classification | Date | Reference KWIC | Draw Desc | Image

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☐ 9. Document ID: US 5489524 A

L9: Entry 9 of 13 File: USPT Feb 6, 1996

US-PAT-NO: 5489524
DOCUMENT-IDENTIFIER: US 5489524 A

TITLE: Chimeric protein that has a human Rho motif and deoxyribonuclease activity

DATE-ISSUED: February 6, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Resnick; Michael A.	Chapel Hill	NC	N/A	N/A
Perkins; Edward L.	Carrborro	NC	N/A	N/A
Chow; Terry	Fleurimont	N/A	N/A	CAX

US-CL-CURRENT: 435/199; 435/193

Full | Title | Citation | Front | Review | Classification | Date | Reference KWIC | Draw Desc | Image

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☐ 10. Document ID: US 5262177 A

L9: Entry 10 of 13 File: USPT Nov 16, 1993

US-PAT-NO: 5262177
DOCUMENT-IDENTIFIER: US 5262177 A

TITLE: Recombinant viruses encoding the human melanoma-associated antigen

DATE-ISSUED: November 16, 1993

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Brown; Joseph P.	Seattle	WA	N/A	N/A
Estin; Charles D.	Bainbridge Island	WA	N/A	N/A
Plowman; Gregory D.	Seattle	WA	N/A	N/A
Rose; Timothy M.	Seattle	WA	N/A	N/A
Hellstrom; Karl E.	Seattle	WA	N/A	N/A
Hellstrom; Ingegerd	Seattle	WA	N/A	N/A
Purchio; Anthony F.	Seattle	WA	N/A	N/A
Hu; Shiu-Lok	Redmond	WA	N/A	N/A
Pennathur; Sridhar	Seattle	WA	N/A	N/A

US-CL-CURRENT: 435/235.1; 424/185.1, 424/199.1, 424/232.1, 435/252.3, 435/252.33, 435/320.1, 435/362, 435/69.3, 530/350 , 536/23.5

Full | Title | Citation | Front | Review | Classification | Date | Reference | KWIC | Draw Desc | Image

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Term	Documents
COMMON.DWPI,EPAB,JPAB,USPT.	1106289
COMMONS.DWPI,EPAB,JPAB,USPT.	521
(8 AND COMMON).USPT,JPAB,EPAB,DWPI.	13

Display 11 Documents, starting with Document: 11

Display Format: Change Format

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Search Results - Record(s) 11 through 13 of 13 returned.

☐ 11. Document ID: US 5141742 A

L9: Entry 11 of 13 File: USPT Aug 25, 1992

US-PAT-NO: 5141742
DOCUMENT-IDENTIFIER: US 5141742 A

TITLE: Vaccines against melanoma

DATE-ISSUED: August 25, 1992

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Brown; Joseph P.	Seattle	WA	N/A	N/A
Estin; Charles D.	Bainbridge Island	WA	N/A	N/A
Plowman; Gregory D.	Seattle	WA	N/A	N/A
Rose; Timothy M.	Seattle	WA	N/A	N/A
Hellstrom; Karl E.	Seattle	WA	N/A	N/A
Hellstrom; Ingegerd	Seattle	WA	N/A	N/A
Purchio; Anthony	Seattle	WA	N/A	N/A
Hu; Shiu-Lok	Redmond	WA	N/A	N/A
Pennathur; Sridhar	Seattle	WA	N/A	N/A

US-CL-CURRENT: 424/186.1; 424/277.1, 435/69.3, 435/70.1, 435/71.1, 435/71.2, 530/350, 530/395, 536/23.5

Full | Title | Citation | Front | Review | Classification | Date | Reference KWIC | Draw Desc | Image

☐ 12. Document ID: US 5093257 A

L9: Entry 12 of 13 File: USPT Mar 3, 1992

US-PAT-NO: 5093257
DOCUMENT-IDENTIFIER: US 5093257 A

TITLE: Hybrid prokaryotic polypeptides produced by in vivo homologous recombination

DATE-ISSUED: March 3, 1992

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Gray; Gregory L.	South San Francisco	CA	N/A	N/A

US-CL-CURRENT: 435/202; 435/220, 435/221, 435/222

Full | Title | Citation | Front | Review | Classification | Date | Reference KWIC | Draw Desc | Image

13. Document ID: US 4870023 A

L9: Entry 13 of 13 File: USPT Sep 26, 1989

US-PAT-NO: 4870023
DOCUMENT-IDENTIFIER: US 4870023 A

TITLE: Recombinant baculovirus occlusion bodies in vaccines and biological insecticides

DATE-ISSUED: September 26, 1989

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Fraser; Malcolm J.	South Bend	IN	N/A	N/A
Rosen; Elliot D.	South Bend	IN	N/A	N/A
Ploplis; Victoria A.	South Bend	IN	N/A	N/A

US-CL-CURRENT: 435/235.1; 435/243, 435/320.1, 435/69.3, 435/69.7, 530/350, 530/820, 530/826, 536/23.1, 536/23.4, 930/10 , 930/220

Full | Title | Citation | Front | Review | Classification | Date | Reference | KWIC | Draw Desc | Image

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Term	Documents
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COMMONS.DWPI,EPAB,JPAB,USPT.	521
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 NEWS  5  Apr 23   Search Derwent WPINDEX by chemical structure
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 NEWS  7  May 07   DGENE Reload
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 NEWS  9  JUL 13   New SDI alert frequency now available in Derwent's
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 NEWS 13  Sep 17   IMSworld Pharmaceutical Company Directory name change
                   to PHARMASEARCH

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               AND CURRENT DISCOVER FILE IS DATED 07 AUGUST 2001
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L1 91 HOMOLOGOUS(10A) RECOMBINATION#(10A) VIVO(10A) PLASMID#

=> s l1 and library

L2 9 L1 AND LIBRARY

=> s l2 and common

L3 0 L2 AND COMMON

=> dup rem l2

PROCESSING COMPLETED FOR L2
L4 5 DUP REM L2 (4 DUPLICATES REMOVED)

=> d l4 1-5 bib ab

L4 ANSWER 1 OF 5 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 1
AN 1995:652042 CAPLUS
DN 123:219560
TI In **vivo** cloning by **homologous recombination** in yeast using a two-**plasmid**-based system
AU Degryse, Eric; Dumas, Bruno; Dietrich, Mireille; Laruelle, Laurence; Achstetter, Tilman
CS Yeast Department, Transgene SA, Strasbourg, 67000, Fr.
SO Yeast (1995), 11(7), 629-40
CODEN: YESTE3; ISSN: 0749-503X
DT Journal
LA English
AB In order to reduce the no. of classical DNA manipulation and ligation steps in the generation of yeast expression plasmids, a series of vectors is described which facilitate the assembly of such plasmids by the more efficient 'recombination in vivo' technique. Two sets of vectors were developed. The first set, called 'expression vectors', contains an expression cassette with a yeast promoter and the PGK terminator sepd. by a polylinker, and an Escherichia coli replicon. Subcloning in these vectors of a DNA fragment generates a 'transfer vector' which is compatible with the second set of E. coli-yeast shuttle vectors. This set of 'recombination vectors' contains a cassette for a functional copy of a gene complementing a host strain auxotrophy or a bacterial gene conferring an antibiotic resistance to the plasmid-bearing host. Plasmid copy nos. can be modulated through the use of URA3 or URA3-d as the selective marker together with an ARS/CEn and the 2 .mu.m replicon. Integration of the cloned DNAs into the yeast linearized replicative vectors occurs by recombination between homologous flanking sequences during transformation in yeast or E. coli. All the vectors contain the origin of replication of phage f1 and allow the generation of single-stranded DNA in E. coli for sequencing or site-directed mutagenesis. The sequence presented has been entered in the EMBL data **library** under Accession No. Z48747.

L4 ANSWER 2 OF 5 MEDLINE DUPLICATE 2

AN 90321590 MEDLINE
DN 90321590 PubMed ID: 2196913
TI The selective isolation of cosmid clones by homologous recombination in Escherichia coli--a cosmid clone containing t complex linkage DNA sequence of mouse was isolated.
AU Chai J H
CS Institute of Genetics, Fudan University, Shanghai.
SO I CHUAN HSUEH PAO. ACTA GENETICA SINICA, (1990) 17 (1) 38-45.
Journal code: A05; 7900784. ISSN: 0379-4172.
CY China
DT Journal; Article; (JOURNAL ARTICLE)
LA Chinese
FS Priority Journals
EM 199008
ED Entered STN: 19901012
Last Updated on STN: 19901012
Entered Medline: 19900828
AB A procedure for the selective isolation of specific cosmid clones by homologous recombination between cosmid clones of genomic **library** and a probe DNA sequence cloned in a plasmid in vivo has been developed. The cosmid **library** was constructed in a rec- host cell strain and packaged into phage particles in vivo. The rec+ host cells containing a DNA sequence used as selection probe cloned in the pUC plasmid were infected by packaged cosmid phage particles. There is no homology between cosmid and the plasmid vectors. After a period of 1-3 hr. for the recombination to take place, the probe **plasmids** were integrated into cosmid, in which the DNA sequence are **homologous** with the probe, by homologous **recombination**. The cosmids are then packaged in **vivo** and transferred into a rec- cell strain. The specific cosmid clones were selected by double antibiotic resistance carried by both vectors. The probe plasmid can be excised by lambda excision enzyme by using superinfection with red+ phage. After packaging in vivo, these cosmid revertants can be identified on Xgal plate. A cosmid clone containing the t complex linkage DNA sequence of mouse was selected by using the procedure above with a probe derived from microdissected metaphase chromosome.

L4 ANSWER 3 OF 5 CAPLUS COPYRIGHT 2001 ACS
AN 1987:472056 CAPLUS
DN 107:72056
TI Syrinx 2A: an improved .lambda. phage vector designed for screening DNA libraries by recombination in vivo
AU Lutz, Charles T.; Hollifield, William C.; Seed, Brian; Davie, Joseph M.; Huang, Henry V.
CS Sch. Med., Washington Univ., St. Louis, MO, 63110, USA
SO Proc. Natl. Acad. Sci. U. S. A. (1987), 84(13), 4379-83
CODEN: PNASA6; ISSN: 0027-8424
DT Journal
LA English
AB The Syrinx 2A phage and .pi.AN13 **plasmid** were designed for screening of DNA libraries by **homologous recombination** in **vivo**. Syrinx 2A carries multiple cloning sites and a recently identified .lambda. gene, rap (recombination adept with plasmid), required for efficient phage-plasmid recombination. A rapid, reliable, and tech. easy method to screen Syrinx 2A libraries, expand the resulting phage-plasmid cointegrates, and subclone plasmid in as little as 2 days is described. Recombination screening allows one specific member of a closely related multigene family to be isolated selectively.

L4 ANSWER 4 OF 5 MEDLINE DUPLICATE 3
AN 86083190 MEDLINE
DN 86083190 PubMed ID: 2934295

TI Isolation of a functional human interleukin 2 gene from a cosmid **library** by recombination in vivo.
AU Lindenmaier W; Dittmar K E; Hauser H; Necker A; Sebald W
SO GENE, (1985) 39 (1) 33-9.
Journal code: FOP; 7706761. ISSN: 0378-1119.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198602
ED Entered STN: 19900321
Last Updated on STN: 19980206
Entered Medline: 19860219
AB A method has been developed that allows the isolation of genomic clones from a cosmid **library** by homologous recombination in vivo. This method was used to isolate a human genomic interleukin 2 (IL2) gene. The genomic cosmid **library** was packaged in **vivo** into lambda phage particles. A **recombination**-proficient host strain carrying IL2 cDNA sequences in a non-**homologous plasmid** vector was infected by the packaged cosmid **library**. After in vivo packaging and reinfection, recombinants carrying the antibiotic resistance genes of both vectors were selected. From a recombinant cosmid clone the chromosomal IL2 gene was restored. After DNA mediated gene transfer into mouse Ltk- cells human IL2 was expressed constitutively.

L4 ANSWER 5 OF 5 MEDLINE DUPLICATE 4
AN 84248071 MEDLINE
DN 84248071 PubMed ID: 6330743
TI Selective isolation of cosmid clones by homologous recombination in Escherichia coli.
AU Poustka A; Rackwitz H R; Frischauf A M; Hohn B; Lehrach H
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1984 Jul) 81 (13) 4129-33.
Journal code: PV3; 7505876. ISSN: 0027-8424.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198408
ED Entered STN: 19900320
Last Updated on STN: 19900320
Entered Medline: 19840822
AB A procedure for selection of specific cosmid clones by homologous recombination between cosmid clones from a **library** and sequences cloned into a plasmid has been developed. Cosmid libraries constructed in a rec- host strain are packaged in vivo into lambda particles. Appropriate aliquots are then introduced into a rec+ host containing the sequence used for selection cloned into a plasmid vector without sequence homology to the cosmid vector. After a short time for recombination, the cosmids are packaged in **vivo**. Cosmids that have taken up the **plasmid** by **homologous recombination** are isolated by plating under conditions selecting for the antibiotic resistance markers carried by both vectors. The recombined cosmids can lose the inserted sequence by another homologous recombination event and, after packaging in vivo, these revertants can be identified on appropriate indicator plates.

=> d l4 3 kwic

L4 ANSWER 3 OF 5 CAPLUS COPYRIGHT 2001 ACS
AB The Syrinx 2A phage and .pi.AN13 **plasmid** were designed for screening of DNA libraries by **homologous recombination**

in **vivo**. Syrinx 2A carries multiple cloning sites and a recently identified .lambda. gene, rap (recombination adept with plasmid), required for efficient. . .

ST phage Syrinx2A DNA **library** screening recombination; plasmid piAN13 DNA **library** screening recombination; DNA **library** screening recombination phage plasmid

IT Recombination, genetic
(homologous, DNA **library** screening by, phage Syrinx 2A and plasmid .pi.AN13 as vectors for)

IT Gene and Genetic element, microbial
RL: BIOL (Biological study)
(rap, for phage-plasmid recombination, in phage vector Syrinx 2A for DNA **library** screening)

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L9: Entry 5 of 13 File: USPT Jan 18, 2000

DOCUMENT-IDENTIFIER: US 6015687 A
TITLE: Apoptosis-modulating proteins, DNA encoding the proteins and methods of use thereof

BSPR:
Apoptosis is a normal physiologic process that leads to individual cell death. This process of programmed cell death is involved in a variety of normal and pathogenic biological events and can be induced by a number of unrelated stimuli. Changes in the biological regulation of apoptosis also occur during aging and are responsible for many of the conditions and diseases related to aging. Recent studies of apoptosis have implied that a common metabolic pathway leading to cell death may be initiated by a wide variety of signals, including hormones, serum growth factor deprivation, chemotherapeutic agents, ionizing radiation and infection by human immunodeficiency virus (HIV). Wyllie (1980) Nature, 284:555-556; Kanter et al. (1984) Biochem. Biophys. Res. Commun. 118:392-399; Duke and Cohen (1986) Lymphokine Res. 5:289-299; Tomei et al. (1988) Biochem. Biophys. Res. Commun. 155:324-331; Kruman et al. (1991) J. Cell. Physiol. 148:267-273; Ameisen and Capron (1991) Immunology Today 12:102; and Sheppard and Ascher (1992) J. AIDS 5:143. Agents that modulate the biological control of apoptosis thus have therapeutic utility in a wide variety of conditions.

BSPR:
Bcl-2 was discovered at the common chromosomal translocation site t(14:18) in follicular lymphomas and results in aberrant over-expression of bcl-2. Tsujimoto et al. (1984) Science 226:1097-1099; and Cleary et al. (1986) Cell 47:19-28. The normal function of bcl-2 is the prevention of apoptosis; unregulated expression of bcl-2 in B cells is thought to lead to increased numbers of proliferating B cells which may be a critical factor in the development of lymphoma. McDonnell and Korsmeyer (1991) Nature 349:254-256; and, for review see, Edgington (1993) Bio/Tech. 11:787-792. Bcl-2 is also capable of blocking of .gamma. irradiation-induced cell death. Sentman et al. (1991) Cell 67:879-888; and Strassen (1991) Cell 67:889-899. It is now known that bcl-2 inhibits most types of apoptotic cell death and is thought to function by regulating an antioxidant pathway at sites of free radical generation. Hockenbery et al. (1993) Cell 75:241-251.

DEPR:
An amino acid sequence comparison of the six known bcl-2 family members (FIG. 6) revealed two regions with considerable sequence identity, namely amino acids 144-150 and 191-199. In an attempt to identify new bcl-2 family members, degenerate PCR primers based on sequences in these regions were designed (FIG. 1) and PCR was performed using human heart cDNA and human B lymphoblastoid cell line (WIL-2) cDNA. PCR was performed using the Hot Start/Ampliwax technique (Perkin Elmer Cetus). The final concentration of the PCR primers and the template cDNA were 4 .mu.M and 0.1-0.2 ng/ml, respectively. The conditions for cDNA synthesis were identical to those for first strand cDNA synthesis of the cDNA library as described below. PCR was performed in a Perkin Elmer Cetus DNA Thermal Cycler according to the method described by Kiefer et al. (1991) Biochem. Biophys. Res. Commun. 176:219-225, except that the annealing and extension temperatures during the first 10 cycles were 36.degree. C. Following PCR, samples were treated with 5 units of DNA polymerase I, Klenow fragment for 30 min at 37.degree. C. and then fractionated by electrophoresis on a 7% polyacrylamide, 1.times. TBE (Tris/borate/EDTA) gel. DNA migrating between

170-210 base pars was excised from the gel, passively eluted for 16 hours with gentle shaking in 10 mM Tris-HCl pH 7.5, 1 mM EDTA (TE), purified by passage over an Elutip-D column (Schleicher and Schuell), ligated to the pCR-Script vector (Stratagene) and transformed into Escherichia coli strain XL1-Blue MRF (Stratagene). Plasmid DNA from transformants (white colonies) containing both the heart and WIL-2 PCR products was isolated using the Magic Miniprep DNA Purification System (Promega), and the DNA inserts were sequenced by the dideoxy chain termination method according to Sanger et al. (1977) Proc. Natl. Acad. Sci. USA 74:5463-5467 (USB, Sequenase version 2.0). DNA sequence analysis of the eleven heart PCR products revealed two sequences identical to bcl-x (Boise et al. (1993) Cell 74:597-608) and ten other sequences unrelated to the bcl-2 family.

DEPR:
To isolate the cdn-1 cDNA, a human heart cDNA library (Clontech) and a WIL-2 cDNA library, constructed as described by Zapf et al. (1990) J. Biol. Chem. 265:14892-14898 were screened using the cdn-1 PCR DNA insert as a probe. The DNA was .sup.32 P-labeled according to the method described by Feinberg and Vogelstein (1984) Anal. Biochem. 137:266-267 and used to screen 150,000 recombinant clones from both libraries according to the method described by Kiefer et al. (1991). Eight positive clones from the WIL-2 cDNA library and two positive clones from the heart cDNA library were identified. Four clones from the WIL-2 cDNA library and two from the heart cDNA library were further purified and plasmid DNA containing the cDNA inserts was excised from the .lambda.ZAPII vector (Stratagene) (FIG. 2). The two longest clones, W7 (2.1 kb) and W5 (2.0 kb) were sequenced and shown to contain the cdn-1 probe sequence, thus confirming their authenticity. The heart cDNAs also encoded cdn-1.

DEPR:
A clone was used to generate recombinant viruses by in vivo homologous recombination between the overlapping sequences of the plasmid and AcNPV wild type baculovirus. After 48 hours post-transfection in insect Spodoptera frugiperda clone 9 (SF9) cells, the recombinant viruses were collected, identified by PCR and further purified. Standard procedures for selection, screening and propagation of recombinant baculovirus were performed (Invitrogen). The molecular mass, on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), of the protein produced in the baculovirus system was compared with the predicted molecular mass of cdn-1 according to the amino-acid sequence.

DEPR:
900,000 clones from a human placenta genomic library in the cosmid vector pWE15 (Stratagene, La Jolla, Calif.) were screened with a 950 bp BgIII- HindIII cDNA probe containing the entire coding region of Cdn-1. The probe was .sup.32 P-labeled according to the method of Feinberg and Vogelstein (1984) Anal. Biochem. 137:266-267. The library was processed and screened under high stringency hybridization and washing conditions as described by Sambrook et al. (1989) Molecular Cloning, 2nd edition, Cold Spring Harbor Laboratory Press. Ten double positive clones were further purified by replating and screening as above. Plasmid DNA was purified using the Wizard Maxiprep DNA Purification System as described by the supplier (Promega Corp., Madison, Wis.) and analyzed by EcoRI restriction enzyme mapping and Southern blotting. The probe used for Southern blotting and hybridization conditions was the same as above.

L9: Entry 10 of 13 File: USPT Nov 16, 1993

DOCUMENT-IDENTIFIER: US 5262177 A
TITLE: Recombinant viruses encoding the human melanoma-associated antigen

BSPR:
FIG. 2 and 2A are diagrammatic representations of the structure of p97 mRNA. The arrangement of the coding region (from the signal sequence to the anchor sequence) and the non-coding region (3'UT) as well as the duplicated domain structure of the p97 precursor (open bar) is indicated. The location of various restriction enzyme recognition sequences are indicated above the mRNA. The relative positions of four cDNA clones are indicated below the mRNA structure. The cDNA clone p97-3a2fl (3a2fl) was isolated from a cDNA library in which the cDNAs were transcribed on oligo(T)-primed p97-enriched mRNAs and cloned in pBR322; whereas, cDNA clones p97-2fl (2fl), p97-1jl (1jl), and p97-10al (10al) were isolated by priming cDNA synthesis with oligonucleotides that encode p97 exon sequences and cloning the resulting cDNA fragments into lambda-gt10. FIG. 2A is a diagrammatic representation of genomic clones B15, H17, B6.6 and E7.7 which were cloned in lambda L47.1.

DEPR:
The monoclonal antibodies directed against p97 described above can be used in a number of ways to facilitate the identification, characterization, cloning and expression of nucleotide sequences which allow for the production of peptides and proteins related to the p97 antigen, in large quantities. For example, the monoclonal antibodies may De used to further characterize the p97 antigen by radiolabeled: all the proteins made by the tumor cell, immunoprecipitating the tumor protein with the monoclonal antibody used to identify the p97 antigen, and fractionating the immunoprecipitated proteins by gel electrophoresis. Protein antigens are identified as distinct bands on the resulting autoradiograph (Brown et al., 1980, J. Biol. Chem. 255:4980-4983). In addition, the monoclonal antibodies directed against p97 can be used to facilitate cloning: as follows: (a) to immunopurify polysomes in order to identify and obtain mRNA transcripts present in the melanoma cell which encode the p97 antigen; (b) to identify clones in a cDNA expression library that express peptides or proteins related to the p97 antigen; (c) to purify the p97 antigen in order to prepare additional monoclonal antibodies or antisera for use in the previous two applications; or (c) to identify cells into which the gene for the p97 antigen has been introduced by transfection.

DEPR:
Early cloning studies concentrated on abundant proteins such as globin and ovalbumin, whose mRNAs often comprised 10 to 50% of total m:RNA. These m:RNAs could be purified to homogeneity by size fractionation, and pure cDNA probes were used to screen libraries of a few hundred clones by colony hybridization. For proteins whose mRNAs comprise 1 of 10% of total mRNA, differential hybridization with two cDNA probes can be used, in which o: of the cDNA probes contains the sequence of interest, and the other is a negative control. Messenger RNAs coding for low-abundance proteins, such as tumor-associated antigens, which may comprise as little as 0.01% of cellular A, are much more difficult to clone, because tens of thousands of clones must be screened, and cDNA probes will not give a specific hybridization signal. Both problems can be alleviated by enriching the mRNA for the sequence of interest.

DEPR:
Another method that may be used in accordance with the invention to clone the

cDNA coding for a tumor-associated antigen such as p97 is to determine a partial or complete amino acid sequence of the antigen and to synthesize an oligonucleotide probe based on the nucleotide sequence deduced from the amino acid sequence. The oligonucleotide may then be used as a primer for cDNA synthesis and as a probe to screen the resulting cDNA library. Accordingly, the melanoma associated p97 protein may conveniently be purified from lysates of melanoma cells by affinity chromatography with a specific monoclonal antibody (Brown, et al., 1982, Nature, London 296:171-173). A nucleotide sequence coding for part of the determined amino acid sequence is then synthesized which can be used as a primer and/or probe. Parts of the amino acid sequence containing amino acid residues coded by a single codon or two codons are most suitable for this purpose. One approach is to synthesize a longer sequence, typically 25 to 60 nucleotides, which represents the most probable coding sequence based upon the known codon usage frequencies in humans. The use of two synthetic oligonucleotides based on different parts of the amino acid sequence facilitates the screening by allowing he to identify spurious positive hybridization signals. Additionally, the use of hybridization conditions that minimize the effect of GC-content on the melting point of DNA hybrids also facilitates the screening. Once a partial cDNA clone has been obtained by this method it may be used as a probe to help obtain a full-length cDNA clone.

DEPR:
Cloning vectors have been developed that allow for expression of the cDNA insert in bacteria. One approach, therefore, which can be used to obtain cDNA clones for tumor-associated proteins such as p97, is to prepare a cDNA library by reverse transcribing the mRNA (enriched or unenriched) isolated from melanoma cells as described above, using oligo(T)-nucleotide primers or the synthetic oligonucleotide primers described above, and to screen such a library with a monoclonal antibody directed against the melanoma associated p97 protein. Clones that contain DNA coding for the epitopes recognized by the monoclonal antibody in the correct orientation and reading frame will express peptides or proteins related to the melanoma associated p97 protein and can be identified by transferring the proteins expressed by the clones to a nitrocellulose filter and incubating the filter with the antibody, followed by development with a labeled anti-immunoglobulin reagent.

DEPR:
Once a recombinant virus or plasmid that is believed to contain a cDNA insert derived from a melanoma associated p97 antigen is identified, the cDNA insert can be used to screen additional libraries in order to identify either full-length clones or else a group of clones that span the full length of the cDNA that codes of p97. The identity of the cDNA cloned can be established by sequence analysis and comparison of the deduced N-terminal amino acid sequence with that determined by direct amino acid sequence analysis of the p97 protein.

DEPR:
The following method allows cloning of DNA using a monoclonal antibody directed against an antigenic determinant that is present only in the native protein and is not present in nascent chains or in protein expressed in bacteria. To this end DNA derived from the human melanoma cell is introduced in mouse L cells by transfection. Subsequently, mouse cells that express melanoma associated p97 antigen are isolated either by using the fluorescence-activated cell sorter or by the immunological identification of colonies that produce p97 related peptides using radiolabeled monoclonal antibodies directed against p97 to detect related peptides on replicas of colonies transferred to polyester cloth filters. Several subsequent rounds of transfection may be required to remove unrelated human DNA sequences. A genomic library is then prepared in a lambda phage vector and screened for clones containing human repetitive sequences which occur in the introns of most genes. Once a genomic clone is identified it can be used as a hybridization probe to identify cDNA clones containing the DNA coding for-p97.

DEPR:
In order to construct a recombinant vaccinia virus expressing the melanoma associated p97 antigen, the cDNA coding sequence can be ligated to the 7.5K promoter of vaccinia virus to form a chimeric gene. This chimeric gene is

flanked by additional vaccinia viral sequence homologous to the viral thymidine kinase gene, which is carried on the plasmid DNA vector. The construction of the chimeric gene involves the use of both natural and synthetic control signals for transcription and translation of the tumor-associated antigen sequence. The chimeric gene is then introduced into vaccinia virus expression vectors through in vivo recombination between the homologous thymidine kinase region present on both the plasmid vector and the vaccinia virus genome. These recombinant viruses containing the chimeric gene are capable of directing the expression of p97 related peptides in an infected host and can be used as components of a vaccine.

DEPR:
Attempts to obtain cDNA clones extending more than 1 kb from the polyadenylation site were unsuccessful, possibly due to a region of high GC content (greater than 80%) with extensive secondary structure. Genomic cloning was used to circumvent this problem. Four overlapping genomic clones were isolated from libraries of lambda L47.1 containing size-fractionated SK-MEL 28 DNA enriched for a specific p97 restriction fragment. These four genomic clones span 28kb and contain the entire coding region of p97 including the regulatory region of the gene. The genomic

DEPR:
Three synthetic oligonucleotides, the sequences of which were based on p97 genomic exon sequences, were used to prime cDNA synthesis on SK-MEL 28 mRNA and the resulting cDNA was cloned into lambda-gt10 as follows: the p97 cDNA was detailed and ligated with a bridger oligonucleotide (AATTCCCCCCCCCCCC) and lambda-gt10 which had been restricted with EcoRI. The bridger oligonucleotide permitted insertion and ligation of the detailed cDNA sequence into the EcoRI site of lambda gt10. The lambda phage was packaged (Grosveld et al., 1981, Gene 13:227-237), and plated on E. coli c.sub.600 rK.sup.- mK.sup.+ hfl. The cDNA libraries in lambda-gt10 were screened for the p97 insert by plaque hybridization (Benton & Davis, 1977, Science 196:180) with genomic exon fragments as probes. Probes were radiolabeled with 32P-TTP (New England Nuclear, 3200 Ci/mmole) by nick-translation to a specific activity of 5-10.times.10.sup.8 cpm/.mu.g. Three overlapping cDNA clones (10a1, 1j1, 2f1) spanning 2,368 nucleotides of the p97 mRNA, including the entire coding region, were identified by using p97 exon specific fragments as probes (FIG. 2).

DEPR:
A search of the amino acid sequence library of the Protein Identification Resource (Release 5.0; Dayhoff et al., 1981, Nature, London 290:8) showed that p97 is strikingly homologous to three members of the transferrin superfamily: human serum transferrin, human lactotransferrin an chicken transferrin (37%-39% homology, see FIG. 4). Since human and chicken transferrin show 50% homology to each other, p97 must have diverged from serum transferrin more than 300 million years ago. p97 has 14 cysteine residues located in homologous positions in each domain. Human transferrin contains all of these cysteines in homologous positions in both domains, while human lactrotransferrin and chicken transferrin lack only two of these cysteine residues (in their C-terminal domains). Unlike p97, these proteins contain 4-7 additional cysteines in their C-terminal mains which have no corresponding member in the N-terminal domain. Human transferrin also contains 2 extra cysteines unique to its N-terminal domain. The positions t st of the disulfides in human serum transferrin, lactotransferrin and chicken transferrin have been determined directly (McGillivray et al., 1982, Proc. Natl. Acad. Sci. U.S.A. 79:2504-2508; Metz-Boutigue et al., 1984, Eur. J. Biochem 145:659-676; Mazurier et al., 1983, Experietia (Basel) 39:135-141; MacGillivray er al., 1983, J. Biol. Chem. 258:3543-3553; Williams et al., 1982, Eur. J. Biochem. 122:297-303; Williams, 1974, Biochem J. 141:745-752). One can thus predict the presence of 7 disulfide bonds in each domain of p97 (see FIG. 5).

DEPR:
Its membership in the transferrin superfamily, its ability to bind iron (Brown et al., 1982, Nature London 296:171-173), and its common chromosomal localization with transferrin and the transferrin receptor (Plowman et al., 1983, Nature, London, 303:70-72; Yang et al., 1984, Proc. Natl. Acad. Sci.

U.S.A. 81:2752-2756) all support a role of p97 in iron transport. The iron binding pocket of transferrin is thought to contain 2-3 tyrosines, 1-2 histidines and a single bicarbonate-binding arginine (Metz-Boutigue et al., 1984, Eur. J. Biochem. 145:659-676). Conservation of these amino acids in p97 support its proposed role in iron metabolism (see FIG. 4). Since p97 is a membrane bound transferrin-like molecule and has no homology with the transferrin receptor (Schneider et al., 1984, Nature, London, 311:675-678), its role in cellular iron metabolism may differ from that provided by circulating serum transferrin and the cellular receptor for transferrin. Expression of the cloned p97 cDNA in eukaryotic cells will allow experimental testing of its functional properties.

DEPR:
The expression plasmid driven by the SV-40 early promoter sv2 was constructed from the cDNA plasmid clone p97a, which is similar to plasmid p97b, except that the entire 3'UT region is utilized (FIG. 6). All cDNA clones were originally isolated from lambda gt10 libraries with synthetic EcoRI-dG (9-17) linkers as previously described. Inserts were excised by EcoRI and subcloned into pEMBL18+ for subsequent propagation and characterization. Clone 10al was subcloned into M13mp18 and an RF form was digested with BamHI and SphI, treated briefly with Exonuclease III, blunted with S1 nuclease, treated with Klenow, and religated. Several plaques were isolated and sequenced, one of which had removed the dG tail and retained 33 bp of the p97 5' untranslated region inserted into the HindIII site of M13mp18. An RF of this subclone (10ala) was used for generating the intact p97 cDNA; otherwise, all fragments were isolated from the plasmid subclones. The 550 bp HindIII-PvuII fragment from 10ala and the 735 bp PvuII-SalI fragment from 1jl were isolated from LMP agarose gels and ligated into pEMBL18+ at the SalI and HindIII sites, generating p5'p97. E7.7 genomic clone in pEMBL18+ was digested to completion with EcoRI and digested partially with SstI, and the 4.5 kb fragment was separated by fractionation through 0.8% LMP agarose. This 4.5 kb 3' fragment was ligated with the 404 bp SstI fragment from 2fl and the 535 bp BamHI-SstI fragment from 1jl into pEMBL18+ at the SalI and EcoRI sites, generating p3'p97. The 1285 bp HindIII-SalI fragment of p5'p97 was then ligated into p3'p97, generating pp97a. The EcoRI-partial HindIII fragment from this clone was inserted into pSV2neo (Southern, et al., 1982, J. Mol. App. Genet. 1:327-341) at the HindIII and EcoRI sites, eliminating the neomycin coding region and SV 40 splice/polyA sequences while retaining the SV40 early promoter and 72 bp enhancer, 33 bp p97 5'UTR, the entire p97 coding region, 3' UTR and 1.4 kb 3' flanking DNA. The resulting plasmid was termed pSVp97a.

DEPC:
(c) cDNA EXPRESSION LIBRARIES

L9: Entry 12 of 13 File: USPT Mar 3, 1992

DOCUMENT-IDENTIFIER: US 5093257 A
TITLE: Hybrid prokaryotic polypeptides produced by in vivo homologous recombination

BSPR:
Recombinant polypeptides have also been modified by fusing recombinant DNA sequences. For example, the signal sequence from a plasmid-derived beta-lactamase was positioned at the amino-terminus of proinsulin through a common restriction site to facilitate the secretion of proinsulin (3).

BSPR:
Two different human alpha interferon DNA sequences have been combined by way of a common restriction site to form a DNA sequence containing sequences from alpha-1 interferon and alpha-2 interferon as described by Weissman (4). The alpha interferons expressed by such fused alpha interferon DNA sequence, however, demonstrated limited biological activity.

BSPR:
Weber, et al., (5) disclose a method for making modified genes by in vivo recombination between DNA sequences encoding an alpha-1 and an alpha-2 human interferon sequence. A linear DNA sequence containing a plasmid vector flanked by the alpha-2 interferon gene on the 5' end and a portion of alpha-1 interferon gene on the 3' end was used to transfect a rec A positive strain of E. coli. Circularization of the linear plasmid by in vivo recombination between the partially homologous interferon gene sequences produced a number of modified interferon genes containing various portions of the alpha-1 and alpha-2 interferon gene sequences. Weber reports that some of these modified alpha interferon genes expressed modified alpha interferons having biological activity similar to unmodified alpha-2 interferons.

DEPR:
Approximately 5.times.10.sup.3 plaques were plated onto E. coli Q359 bacteria on LB plates containing 0.5% starch to screen for alpha amylase activity. These plates were exposed to iodine vapors to stain the starch (16). Five plaques which were surrounded by clear halos were found in the B. licheniformis library. Three such plaques were found in the B. stearothermophilus library. DNA was prepared from each one of the amylase positive B. licheniformis phage (designated .lambda.-amy-BL) and the B. stearothermophilus phage (designated .lambda.-amy-BS).

DEPR:
The 14 putative hybrids showed a variety of hybridization patterns. For example hybrids 1, 2, 3, and 4 hybridized to probe 1 but not to the other (more 3') probes 2-8. This indicated crossover between sequences corresponding to probes 1 and 2. In another example, hybrid 9 hybridized to probes 1-4 but not to the more 3' probes 5-9. This indicated a crossover between the sequences corresponding to probes 4 and 5. As shown in Table 1 other crossover regions were similarly identified in the remaining hybrids. In other experiments (not shown) hybrids with crossovers between any pair of probe regions were found although with highly variable frequencies, e.g. crossovers between probes 1 and 2 were very common (about 20%) whereas crossovers between probe regions 2 and 3 were quite rare (about 1%).